BISPECIFIC ASYMMETRIC HETERODIMERS COMPRISING ANTI-CD3 CONSTRUCTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 13/941,449, which claims the benefit of U.S. Application Ser. No. 61/671,640, filed Jul. 13, 2012; and U.S. Application Ser. No. 61/845,948, filed Jul. 12, 2013, which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. SAID ASCII copy, created on Jan. 24, 2019, is named 39916US_CRF_sequencelisting.text, and is 859,000 bytes in size.

FIELD OF THE INVENTION

[0003] The field of the invention is the rational design of multispecific scaffolds comprising a CD3 binding domain for custom development of biotherapeutics.

BACKGROUND OF THE INVENTION

[0004] In the realm of therapeutic proteins, antibodies with their multivalent target binding features are excellent scaffolds for the design of drug candidates. Advancing these features further, designed bispecific antibodies and other fused multispecific therapeutics exhibit dual or multiple target specificities and an opportunity to create drugs with novel modes of action. The development of such multivalent and multispecific therapeutic proteins with favorable pharmacokinetics and functional activity has been a challenge.

[0005] The immune system of both humans and animals include two principal classes of lymphocytes: the thymus derived cells (T cells), and the bone marrow derived cells (B cells). T cells exhibit immunological specificity and are directly involved in cell-mediated immune responses (such as graft rejection). T cells act against or in response to a variety of foreign structures (antigens). In many instances these foreign antigens are expressed on host cells as a result of infection. However, foreign antigens can also come from the host having been altered by neoplasia or infection.

[0006] T cell activation is a complex phenomenon that depends on the participation of a variety of cell surface molecules expressed on the responding T cell population. For example, the antigen-specific T cell receptor (TcR) is composed of a disulfide-linked heterodimer, containing two clonally distributed, integral membrane glycoprotein chains, alpha and beta (α and β), or gamma and delta (γ and δ), non-covalently associated with a complex of low molecular weight invariant proteins, commonly designated as CD3.

SUMMARY OF THE INVENTION

[0007] Provided herein are multispecific heteromultimers comprising a CD3 binding domain. In an embodiment is provided a bispecific asymmetric heterodimer comprising anti-CD3 constructs.

[0008] Provided herein are isolated multispecific heteromultimer constructs comprising: a first polypeptide construct comprising a first heavy chain polypeptide and a CD3 binding polypeptide construct that binds to a CD3 complex on at least one CD3 expressing cell; a second polypeptide construct comprising a second heavy chain polypeptide which is different from said first heavy chain polypeptide, and an antigen binding polypeptide construct that binds to a target antigen on at least one B cell; wherein: at least one of said CD3 binding polypeptide construct and said antigen binding polypeptide construct comprises a single chain Fv region; wherein said multispecific heteromultimer construct simultaneously engages said at least one B cell and said at least one CD3 expressing cell such that the CD3 expressing cell is activated, thereby inducing killing of the B cell; and said first and second heavy chain polypeptides form a heterodimeric Fc region comprising a variant immunoglobulin CH3 region comprising at least one amino acid mutation that promotes the formation of said heterodimeric Fc with stability at least comparable to a native homodimeric Fc, and with purity such that when said multispecific heteromultimer construct is coexpressed from a stable mammalian cell in an expression product, said expression product comprises at least about 70% of said multispecific heteromultimer, and less than 10% monomers or homodimers of said first or second polypeptide constructs. In certain embodiments, said stable mammalian cell is transfected at least a first DNA sequence encoding said first polypeptide construct and at least a second DNA sequence encoding said second polypeptide construct in a pre-determined ratio of 1:1. In certain embodiments, the first or second polypeptide construct is devoid of at least one of immunoglobulin light chain, and immunoglobulin first constant (CH1) region.

[0009] In certain embodiments are the isolated heteromultimer constructs described herein, wherein the heterodimer Fc region comprises a variant CH2 domain or hinge comprising amino acid modifications that prevents functionally effective binding to all the Fcgamma receptors. In some embodiments is provided the isolated multispecific heteromultimer described herein, wherein wherien said variant CH2 domain or hinge comprising amino acid modification also prevents functionally effective binding to complement proteins (C1q complex). In an embodiment is the isolated multispecific heteromultimer described herein, wherein the heterodimer Fc region comprises a variant CH2 domain or hinge comprising amino acid modifications that enhance binding to the FcγRIIb receptor.

[0010] In an embodiment is provided an isolated multispecific heteromultimer construct comprising: a first polypeptide construct comprising a first heavy chain polypeptide and a CD3 binding polypeptide construct that binds to a CD3 complex on at least one CD3 expressing cell; a second polypeptide construct comprising a second heavy chain polypeptide which is different from said first heavy chain polypeptide, and an antigen binding polypeptide construct that binds to a target antigen on at least one B cell; wherein: at least one of said CD3 binding polypeptide construct and said antigen binding polypeptide construct optionally comprises a single chain Fv region; said first and second heavy chain polypeptides form a heterodimeric Fc region comprising a variant immunoglobulin CH3 region comprising at least one amino acid mutation that promotes the formation of said heterodimeric Fc, wherein: said heterodimeric Fc is formed with stability at least comparable to a native homodimeric Fc, and said heterodimeric Fc is formed with purity such that when said multispecific heteromultimer construct is coexpressed from a mammalian cell in an expression